Production and Characterization of Antibodies Against Human Tyrosinase

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Proteins mapping at different loci are involved in melanogenesis and share several characteristic structural features (*b* locus, *c* locus, and *slaty* locus products). We describe a method to produce specific antibodies against human tyrosinase, the product of the *c* locus. Mouse L cells transfected with a human tyrosinase cDNA were used to generate antibodies by immunization of syngeneic C3H mice. These antibodies were able to precipitate the tyrosinase glycoprotein from both melanocytic cells and transfectants expressing tyrosinase. In contrast, transfectants expressing the related but distinct *b* locus protein (gp75 or TRP-1) did not react with these antibodies. In most cases, tyrosinase enzymatic activity could be precipitated and recovered in immune complexes, but one antibody response blocked tyrosinase activity. Immunostaining with anti-tyrosinase antibodies revealed an intracellular granular pattern in tyrosinase transfectants and melanocytic cells, but not transfectants expressing the *b* locus protein. This approach provides a general method to produce specific antibodies against tyrosinase, other members of the tyrosinase family of proteins, and potentially any other differentiation antigen. *Key words: pigmentation/transfectants/melanin.*
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A number of genes have been implicated in the regulation of pigmentation in mammals [1]. The best characterized of these genes, located at the *c* locus, encodes tyrosinase (EC 1.14.18.1) [2]. Tyrosinase is a membrane glycoprotein that is specifically expressed in melanocytes. A related glycoprotein, gp75 (the product of the *b* locus, also called TRP-1), is also specifically expressed by cells of the melanocytic lineage and is localized to melanosomal membranes [3]. Recently, other potential members of this family have been identified, designated TRP-2 and Pmel 17 [4,5]. Tyrosinase, gp75 (or TRP-1), and TRP-2 display a number of structural similarities. These three gene products: 1) are homologous (between 40% and 45% at the amino acid level) but distinct from one another; 2) have nearly identical molecular masses; 3) have highly conserved cysteine residues that are implicated in tertiary structure; 4) include putative copper binding sites that could be involved in enzymatic activities; and 5) contain transmembrane domains at comparable positions. These observations have led to the concept of a broader "tyrosinase-related" family of proteins [6]. Antibodies have played a crucial role in identifying and characterizing these genes and their products. Most of these antibodies were derived before the family of tyrosinase-related proteins was identified. Antibodies against melanocyte antigens have been generated by immunizing animals with whole cells (most frequently melanoma cells), purified proteins, or peptides. Based on the similarities of these proteins, antibodies against one member of the tyrosinase-related protein family frequently react with other distinct family members. As a result, specificities of antibodies against tyrosinase, TRP-1, and other members have often remained incompletely defined. For instance, antibodies against tyrosinase were used to isolate the mouse TRP-1 gene, the mouse TRP-2 gene, and the human Pmel 17 gene [4–7]. The isolation of genes that encode these proteins has permitted the generation of polyclonal antisera against defined peptide sequences that are distinct for each protein member of the tyrosinase family [8]. This approach has provided valuable reagents. For instance, recently the product of the TRP-2 gene has been identified by raising antisera against synthetic peptides corresponding to unique sequences of TRP-2 [4]. Transfected cell lines expressing these different gene products provide another way to generate potentially unambiguous reagents. In this report, we describe the use of mouse fibroblasts transfected with human tyrosinase cDNA to generate antibodies against tyrosinase that do not cross-react with TRP-1 by immunization of syngeneic C3H mice. We have characterized the specificity of antibodies produced by this method against cells of the melanocytic lineage and non-melanocytic cells expressing human tyrosinase and gp75/TRP-1.

MATERIALS AND METHODS

Cells and Cell Lines Human melanoma cell lines (SK-MEL-30, SK-MEL-188, SK-MEL-204, clone 1-5 of SK-MEL-131, and clone 22 of SK-MEL-23) and the human melanocyte culture FS120 were cultured as previously described [9]. The human renal cell carcinoma SK-RC-28 [10] and the mouse melanoma cell line B16 [11] have been reported. L-TY cells are mouse fibroblast L cells stably transfected with the human tyrosinase cDNA BB-TY1, and cultured as described [12]. L-GP cells were generated in a similar manner by transfection of a full-length cDNA encoding gp75 (TRP-1) into L cells (S. Vijayasaradhi *et al,* manuscript in preparation). All cell lines were grown in modified Eagle's medium (MEM) supplemented with 10% fetal bovine serum (FBS) and non-essential amino acids, antibiotics, and L-glutamine.

Antibodies The monoclonal antibody (MoAb) TA99, directed against gp75 (TRP-1), has been reported elsewhere [13]. Polyclonal rabbit serum raised against purified murine tyrosinase was a generous gift from Dr. B. B. Fuller, University of Oklahoma [14]. Rabbit anti-mouse immunoglobulin (Ig) conjugated to peroxidase for enzyme-linked immunoassay (ELISA) screenings was from Sigma Chemical Co. (St. Louis, MO). Unlabeled and

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Abbreviation: L-DOPA, L-dihydroxyphenylalanine.

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Table I. Characterization of Sera from Mice Immunized with Transfectants Expressing Human Tyrosinase

Method	Mouse Serum[a]							
	M1	M2	M3	M4	M5	M6	M7	M8
Immunoprecipitation of a 70–75-kDa band[b]	−	+	+	−	−	+	−	+
Precipitation of tyrosinase activity[c]	ND[d]	+	+	ND	ND	−	−	+
Immunoreactivity with L-TY fibroblasts[e]	−	+	+	−	−	+	−	+
Immunoreactivity with L-GP fibroblasts[e]	−	−	−	−	−	−	−	−

[a] M1 to M8 are sera from eight different mice, each of which received four immunizations with L-TY transfectant cells as described in *Materials and Methods.*
[b] Immunoprecipitation was from ^{35}S-methionine–labeled L-TY transfectants or human melanoma cell lines.
[c] Assay by *in situ* melanin formation.
[d] ND, not done.
[e] Assay by ELISA and indirect immunofluorescence.

fluorescein-conjugated rabbit anti-mouse or goat anti-rabbit Ig were purchased from Cappel Organon Technika Co. (Westchester, PA).

Generation and Absorption of Anti-Tyrosinase Sera Six-week-old female C3H mice were initially injected intraperitoneally (ip) with 5×10^6 L-TY cells mixed in complete Freund's adjuvant followed by a second immunization with L-TY cells in incomplete Freund's adjuvant 2 weeks later. The third and fourth immunizations (at 2-week intervals) were performed using 5×10^6 L-TY cells lysed in NP-40 (at 10^7/ml) detergent buffer (150 mM NaCl, 10 mM Tris pH 7.6, 2 mm ethylenediamine tetraacetic acid [EDTA], 1% NP-40) and mixed with equal volumes of incomplete Freund's adjuvant. Three days after the fourth immunization, mice were bled and sera were analyzed by ELISA (see below). Sera were obtained from sero-positive mice. In some instances, sera were absorbed with non-transfected L cells to remove non-specific reactivity against L-cell components. For these absorptions, a cell lysate of 30×10^6 L cells in phosphate-buffered saline (PBS) containing 1% (v/v) NP40 was prepared. PBS containing bovine serum albumin 1% (w/v) was used to block strips of nitrocellulose (5 mm × 100 mm) for 1 h. These membranes were then incubated with L cell extracts for 1 h at room temperature. Immune sera (0.5 ml) were then absorbed on the membranes for 1 h before use at indicated dilutions.

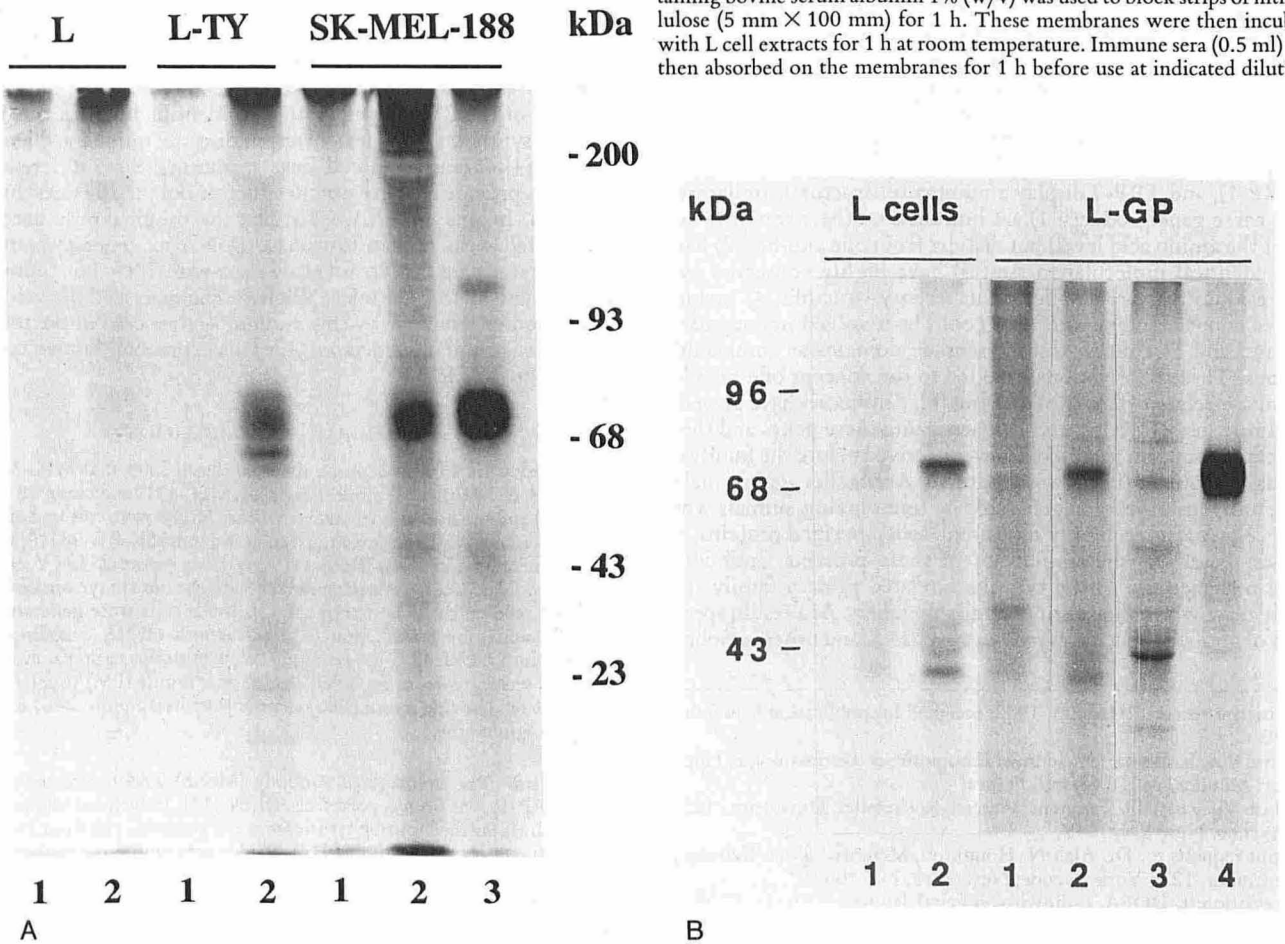

Figure 1. *A)* Specificity of anti-tyrosinase sera. Immunoprecipitation with M3 serum of cell lysates from L cells, L-TY cells, or pigmented SK-MEL-188 melanoma cells labeled with ^{35}S-methionine. Lysates were immunoprecipitated with preimmune sera *(lanes 1), anti-tyrosinase M3 immune sera (lanes 2),* or MoAb TA99 against gp75 *(lane 3).* Molecular weight markers are indicated in kDa. *B)* Anti-tyrosinase sera do not recognize the *brown* protein. Immunoprecipitations of cell lysates from L cells or L-GP cells (transfected with gp75 cDNA), labeled with ^{35}S-methionine. Lysates were immunoprecipitated with preimmune sera *(lanes 1),* control rabbit anti-tyrosinase serum *(lanes 2),* M3 anti-tyrosinase immune sera *(lane 3),* or MoAb TA99 against gp75 *(lane 4).* Molecular weight markers are indicated in kDa.

Immunofluorescence Assays Cultured cells (10^4) were grown on Lab-tek 8 glass tissue culture chamber slides (Nunc, Inc., Naperville, IL) overnight in a humidified incubator at 37°C. Cells were fixed with 2% paraformaldehyde for 10 min at room temperature and permeabilized with methanol for 2 min at −20°C. Non-specific binding was blocked by incubating the slides with PBS containing 2% gamma globulin-free (GG-free) FBS for 1 h at room temperature. Test sera were added to the wells at the indicated dilutions and incubated for 1 h. The wells were washed 3 times with PBS containing 2% GG-free FBS and incubated with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse Ig (1 : 100) or FITC-conjugated rat anti-rabbit (1 : 50) Ig as applicable. Slides were washed twice in PBS containing 2% GG-free serum and mounted in PBS-glycerol containing *p*-phenylenediamine (Sigma). The cells were viewed and photographed with a Nikon Optiphot microscope.

Metabolic Labeling and Immunoprecipitations Cells (5×10^6) were preincubated for 3 h at 37°C in methionine-free medium with 2% dialyzed FBS. The cells were then metabolically labeled for approximately 18 h with 200 μCi ^{35}S-methionine (specific activity 1140 Ci/mmol with a concentration of 7.9 mCi/ml) (New England Nuclear, Boston, MA) in 3 ml of the same medium in 25-cm^2 flasks. Cells were trypsinized and washed three times with ice-cold PBS prior to solubilization (at 10^7/ml) in 150 mM NaCl, 10 mM Tris, pH 7.6, 1% NP-40, 2 mM EDTA for 30 min on ice. Insoluble material was removed by centrifugation at 10,000 × *g* for 10 min. Radioactive counts that were precipitated with trichloroacetic acid (5×10^6) were used for each immunoprecipitation. After preclearing twice with *Staphylococcus aureus* (Staph A), lysates were incubated for 30 min with 3 μl of primary antibody at 4°C. Immune complexes were recovered on 30 μl protein A-bound agarose beads (Pharmacia, Piscataway, NJ), and were extensively washed in 1 × TNEN (Tris 10 mM, NaCl 15 mM, EDTA 5mM, 1% NP-40). Samples were eluted and reduced in Laemmli buffer [15] and analyzed on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels. Radioactive bands were revealed by fluorography.

Tyrosinase Activity Assays Cell extracts were homogenized in PBS-1% Triton X-100 (TX100) and centrifuged at 4°C to remove insoluble material. Protein concentrations were determined by Bradford's method using a commercial kit (BioRad, Richmond, CA). Tyrosinase activity was assayed in the extracts by the method of Pomerantz, with slight modifications as previously reported [12] using ^{3}H-tyrosine [16]. Alternatively, tyrosinase activity was recovered after immunoprecipitation and evaluated in pellets: 30 μg unlabeled total protein was incubated with various amounts of antibody (5–50 μl) in a final volume of 300 μl for 1 h at 37°C. The immune complexes were collected by addition of 15 μl of a suspension of Staph A cells (50% w/v in PBS-TX100) and incubation at 37°C for 30 min. The immunoprecipitates were centrifuged and washed three times with PBS-TX100. After addition of 0.2% (w/v) L-dihydroxyphenylalanine (L-DOPA) in phosphate buffer, the reactions were incubated at 37°C for an additional 30 min. Melanin formation was visualized and photographed after centrifugation of pellets. Additional competition assays were performed as follows: whole cell lysates (30 μg of total protein) were incubated with 50 ml of a test serum. Increasing amounts of (5–50 μl) of a second test serum were added to the reaction in a final volume of 300 μl. The assay was then executed as described above.

RESULTS

Human Tyrosinase Expressed in Mouse Fibroblasts is Immunogenic when Injected into Syngeneic Mice We investigated whether specific antibodies against human tyrosinase could be produced using transfected cells. Eight C3H mice (H-2^k haplotype) were immunized with syngeneic L cells (also H-2^k) transfected with human tyrosinase cDNA (L-TY cells). Despite the substantial homology in primary amino acid sequences between mouse and human tyrosinase (83%) [12], high antibody titers ($> 10^{-4}$) were detected against L-TY cells in sera from four of eight immunized mice by ELISA after four immunizations (Table I). No reactivity against L-TY was detected by ELISA in preimmune or normal mouse sera. After three immunizations (every 2 weeks over 6 weeks), ELISA titers against L-TY ranged from 5×10^{-3} to 4×10^{-3}, and a fourth immunization at 10 weeks boosted titers to $> 10^{-4}$. In all positive mice, antibodies were detected against the L cell parental cell line, despite their syngeneic origin, but always at a significantly lower titer than against L-TY cells (10^{-2}).

Sera were used to immunoprecipitate cell lysates of the pigmented human melanoma cell line SK-MEL-188, the non-pigmented melanoma SK-MEL-131 (clone 1-5), and the tyrosinase transfectants L-TY cells. Sera from animals immunized with L-TY cells were capable of precipitating a single broad band of approximately 70–80 kilodaltons (kDa) from L-TY and SK-MEL-188 cell lysates (Table I; a representative immunoprecipitation using M3 serum is shown in Fig 1*A*). A thin band of lower mass, approximately 63 kDa, was also detected in L-TY transfectants (probably representing an intermediate product in tyrosinase biosynthesis) (Fig 1*A*). In contrast, no specific bands were precipitated from non-transfected L cells (Fig 1*A*) or from tyrosinase-negative, amelanotic melanoma SK-MEL-131 (clone 1-5) cells (data not shown). For comparison, MoAb TA99 which recognizes the TRP-1/gp75 glycoprotein, was used to immunoprecipitate the same panel of cell lysates. As expected, TA99, reacted with TRP-1 expressed by pigmented human melanoma cells (Fig 1*A*) and with transfected L-GP cells expressing a full-length human TRP-1 cDNA (Fig 1*B*). MoAb TA99 did not react with L-TY cells and immunodepletion experiments confirmed that TA99 did not deplete the 70–80-kDa band precipitated by M3 anti-tyrosinase sera (data not shown). None of the anti-tyrosinase antibodies (M2, M3, M6, and M8) reacted with gp75$^+$, tyrosinase-L-GP cells by immunofluorescence assays (Table I) and M3 serum did not precipitate gp75/TRP-1 from L-GP cells (Fig 1*B*).

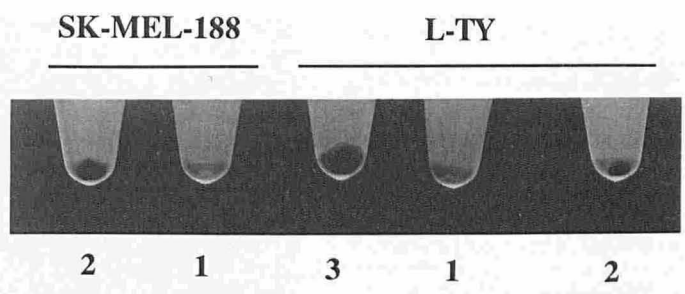

Figure 2. Anti-tyrosinase sera precipitate tyrosinase activity. SK-MEL-188 cells or L-TY cells were lysed in 1% NP40-PBS buffer and immunoprecipitated with preimmune mouse sera *(lanes 1)*, anti-tyrosinase M8 sera *(lanes 2)*, or positive control rabbit anti-tyrosinase serum *(lane 3)*. Immunoprecipitates were recovered on Staph-A cells and melanin formation was visualized after incubation with L-DOPA at 37°C. A black pellet indicates *in situ* melanin formation.

Anti-tyrosinase Antibodies Precipitate Tyrosinase Activity To further test the specificity of immune sera, we analyzed tyrosinase activity precipitated from cell lysates. Methods to characterize antibodies against tyrosinase, using immunodepletion of tyrosinase activity, can lead to ambiguous results. For instance, we have found that control sera or monoclonal antibodies often cause up to 30% non-specific inhibition (a value frequently taken as positive) of enzymatic activity of tyrosinase. For this reason, we chose to analyze both depletion and recovery of tyrosinase activity in the immune complexes.

Table II. Immunoprecipitation of Tyrosine Hydroxylase Activity

Precipitating Serum	Tyrosine Hydroxylase Activity (cpm/min/mg protein)[a]	
	Supernatant	Pellet
Normal mouse serum	4275	535
Rabbit anti-tyrosinase serum[b]	1024	4345
Mouse M3 serum	88	8454
Mouse M6 serum	32	289
Mouse M8 serum	192	7612
Mouse M8 serum followed by normal mouse serum	0	4923
Mouse M8 serum followed by M6 serum	198	544

[a] Assayed using the method of Pomerantz [16]. Experiments were performed three times and gave similar results. Data are from one experiment.
[b] Kindly provided by B. Fuller [14].

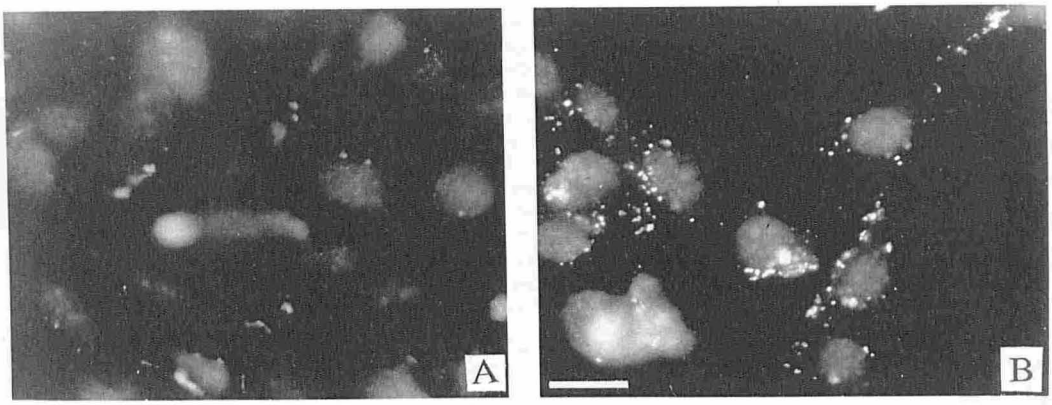

Figure 3. Immunoreactivity of anti-tyrosinase sera against L-cell transfectants. Immunostaining of L cells *(A)*, or L cells transfected with cDNA encoding human tyrosinase, using the anti-tyrosinase serum M3 (dilution 1/1000), and revealed with rabbit anti-mouse FITC second antibody. Exposure time for photomicrograph in (A) (untransfected L cells) was four times longer than for (B) to obtain cell outlines. *Bar,* 20 μM.

Cell lysates of SK-MEL-188, L cells, L-TY, SK-MEL-131 (clone 1-5), or the mouse melanoma line B16 were precipitated with 5, 10, or 25 ml of sera from immunized mice. Control immunoprecipitations included lysates precipitated with MoAb TA99 or preimmune serum. Tyrosinase activity recovered in immunoprecipitates was visualized by formation of the pigment melanin after addition of L-DOPA substrate (for dopa oxidase activity) (Fig 2) and by tyrosine hydroxylase assays (Table II). No pigment was observed in immunoprecipitates of lysates from L cells or the amelanotic melanoma SK-MEL-131 (clone 1-5) (data not shown), or in immunoprecipitates using preimmune serum (Fig 2) or MoAb TA99 (data not shown). In contrast, sera of three of four immune mice (mice M2, M3, and M8) were able to precipitate tyrosinase activity from SK-MEL-188 and L-TY cells (Fig 2). Interestingly, one serum (from mouse M6), although capable of precipitating a 70–80-kDa protein and depleting tyrosinase activity from supernatants (Tables I and II), failed to precipitate any detectable tyrosinase activity in either dopa oxidase or tyrosine hydroxylase assays (Table II and Fig 2).

We postulated that M6 serum might alter or interfere with the catalytic site of tyrosinase. To test this hypothesis, M8 serum was used to immunoprecipitate tyrosinase from L-TY cell lysates and then incubated with either M6 serum or control serum (Table II). M8 serum completely depleted tyrosinase activity from cell lysates and activity could be completely recovered in the immunoprecipitate. However, addition of M6 serum to M8 immunoprecipitates inhibited most tyrosine hydroxylase activity (Table II) and abrogated dopa oxidase activity (not shown). The results indicated that M6 serum could deplete tyrosinase activity without recovery of enzymatic activity in precipitates, and that M6 could inhibit tyrosinase activity precipitated by M8 serum. Thus, M6 antibodies reacted with the tyrosinase molecule but inhibited tyrosine hydroxylase activity and the catalytic formation of pigment, confirming the postulate that M6 serum contained antibodies that reacted with or induced conformational changes in an active catalytic site of tyrosinase.

Table III. Antibody Specificities[a]

Cell Line	Anti-tyrosinase Serum	MoAb TA99	Cell Description
L cells	−	−	Mouse fibroblasts
L-GP	−	+	L cells transfected with gp75 cDNA
L-TY	+	−	L cells transfected with tyrosinase cDNA
SK-MEL-23	+	+	Pigmented melanoma
SK-MEL-188	+	+	Pigmented melanoma
SK-MEL-30	+	+	Pigmented melanoma
SK-MEL-131	−	−	Amelanotic melanoma
SK-MEL-204	−	−	Amelanotic melanoma
SK-RC-28	−	−	Renal cell carcinoma
FS 120	+	+	Normal foreskin melanocytes

[a] Cells were stained by indirect immunofluorescence as described in *Materials and Methods.* +, granular cytoplasmic staining; −, no staining.

Immunostaining with Anti-Tyrosinase Antibodies When absorbed anti-tyrosinase sera were used at a dilution of 1/1000, strong intracellular staining was visible on fixed and permeabilized tyrosinase-transfected L cells and pigmented melanoma cells but not parental L cells. Staining was localized to perinuclear and cytoplasmic granules in L-TY cells (staining with M3 serum is shown in Fig 3). Table III presents the results of specificity testing against a panel of cell lines. Two non-pigmented melanoma cell lines, SK-MEL-131 and SK-MEL-204, and a renal cell carcinoma cell line, SK-RC-28, that expressed no tyrosinase activity and no tyrosinase mRNA by Northern blots (data not shown), did not react with antibodies. In contrast, three pigmented melanoma cell lines and cultured melanocytes, which were known to express high levels of tyrosinase activity and were deeply pigmented, displayed intense cytoplasmic granular staining (Table III and Fig 4*B* showing SK-MEL-23).

Staining with anti-tyrosinase sera was compared with staining of MoAb TA99 against TRP-1 in the panel of cell lines (Table III). Specificity was confirmed using L cells transfected with a full-length cDNA encoding human TRP-1. L cells expressing TRP-1 stained intensely with MoAb TA99 but did not react with antibodies against tyrosinase (Table III). This result supports the previous immunoprecipitation data showing that anti-tyrosinase did not cross-react with the homologous TRP-1 glycoprotein. Antibodies against tyrosinase generally stained fewer cytoplasmic granules than did MoAb TA99 (see Fig 4). Tyrosinase appeared to be expressed in only a subset of intracellular granules that expressed TRP-1.

DISCUSSION

The biosynthesis of tyrosinase that we have expressed in mouse L cell transfectants has not been fully analyzed, partly due to the lack of specific antibodies against human tyrosinase. However, the data presented in this report, together with our previous observations, show that the enzyme processed in the L cells is: a) enzymatically active [12], b) immunogenic, and c) capable of inducing antibodies that specifically recognize and precipitate a native form of the molecule.

The application of specific transfectants has allowed the direct identification and evaluation of the protein of interest. Transfectants have been useful in determining the specificity of serologic responses of the mouse to human tyrosinase. Most antibodies did not inhibit tyrosinase catalytic activity, but at least one mouse generated antibodies that blocked tyrosinase activity. The melanosomal protein gp75 or TRP-1 is considerably more abundant than tyrosinase [17], which might explain why antibodies initially thought to be against tyrosinase were actually directed against TRP-1, particularly when whole cells were used as immunogens. For example, in experiments using single transfectants expressing the murine *b* locus cDNA, Shibahara *et al* recently showed that the MoAb TMH-1 reacts only with the *b* protein and not with tyrosinase as originally postulated [18]. Rabbit anti-hamster polyclonal antibodies gener-

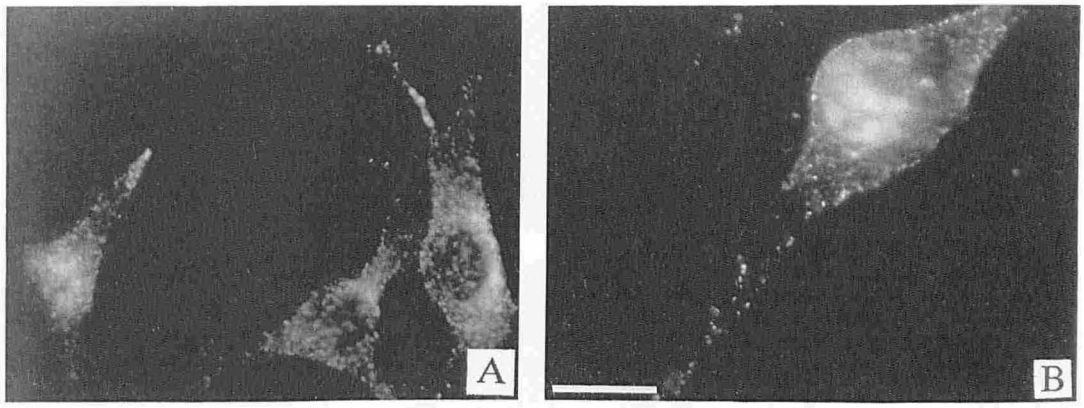

Figure 4. Immunoreactivity of anti-tyrosinase sera against melanocytic cells. Immunostaining of human melanoma cell line SK-MEL-23. Cells were stained with MoAb TA99 against human TRP-1 *(A)* or mouse anti-tyrosinase sera *(B)*. Both anti-tyrosinase serum and TA99 stained cytoplasmic vesicles. *Bar*, 20 μM.

ated by Halaban and Pomerantz [19] have been valuable reagents, allowing the isolation of a cDNA clone encoding human tyrosinase [2]. However, the same polyclonal serum also cross-reacted with other gene products, such as the recently described TRP-2 protein, as illustrated by the isolation of the partial cDNA clone 5A by I. J. Jackson [20]. Similarly, upon initial screening of a human melanocyte cDNA library with the rabbit anti-hamster tyrosinase antisera, Kwon *et al* isolated the unique cDNA designated Pmel 17-1, whose product is not yet formally identified (but may be the *silver* locus product) [5]. The most notable series of antibodies against the tyrosinase family proteins has been generated by Hearing and coworkers. Using peptides with distinct and defined sequences, they have generated antibodies specifically against tyrosinase, TRP-1, and most recently the protein encoded by the TRP-2 gene.

It is interesting that the amino acid sequence differences between mouse and human tyrosinase (<20%) were sufficient to generate high affinity (by virtue of the ability to immunoprecipitate) and specific antibody responses. As pointed out by Hearing and Jimenez [6], tyrosinase has been relatively non-immunogenic (perhaps in part due to its low abundance and its conservation of sequences between mouse and human). However, the application of L cells as a source of antigen in syngeneic mice has allowed the generation of antibodies against this poorly immunogenic molecule. The sera produced by this method should allow more detailed analysis of transfected cells, and help to understand the biosynthesis and cellular localization of tyrosinase in transfectants.

Both tyrosinase and TRP-1 are melanosomal transmembrane glycoproteins. In melanoma cells and melanocytes, the granules stained by anti-tyrosinase serum appeared to represent a sub-population of granules stained by MoAb TA99. In melanocytic cells, these vesicles presumably represent melanosomes. In the case of L-cell transfectants, the nature of these vesicles remains uncertain, although our previous data showed that melanin produced in transfectants was packaged into membrane-bound vesicles [12]. It is reasonable to assume that tyrosinase was trafficked to the same vesicle population that packaged melanin. The ability of transfectants to support the stable production of pigment remains a mystery, as does the identity of vesicles where pigment is synthesized. The observation that only a subset of vesicles in melanoma cells and melanocytes stained with the anti-tyrosinase antibodies, compared to localization of TRP-1, could reflect a difference in half-life of the two proteins (10–12 h for tyrosinase versus 18–24 h for gp75). Differences in tyrosinase activities between distinct cells could reflect rates of synthesis, processing, and degradation. As tyrosinase is transported through the endoplasmic reticulum or the *trans* Golgi network, it is possible that an inactive form of the enzyme or a precursor polypeptide might not be detected by the antibodies that we have produced. For instance, this could reflect differences in processing of human tyrosinase in L cells versus human melanocytic cells. The existence of unambiguous antibodies will allow more careful analysis of the biosynthesis of tyrosinase in transfected L cells as well as in other cell types that are potential recipients for expression of tyrosinase.

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